EPA Reviewer: Irving Mauer, Ph.D. Date: _____
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: _____
Toxicology Branch, HED (7509C)

TXR NO.: 0051042

# DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella typhimurium* and *Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2] OECD 471, 472

DP BARCODE: D287883 SUBMISSION CODE: S621030

P. C. CODE: 288202 (9999-inert) TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): CGA-263208 (N-phenylguanidine, Batch No. EA168376, 95.2% a.i.-- a plant metabolite of CGA 219417)

SYNONYMS: None.

CITATION: Hertner, Thomas (1994). CGA-263208 Technical: Final Report. *Salmonella* and *Escherichia* Mutagenicity Test, performed at Genetic Toxicology of Novartis Crop Protection AG, Basle (SWITZERLAND), Basel Study No. 943052 (Novartis No. 530-94), dated July 18, 1994. MRID 45353506. Unpublished.

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays in bacteria (MRID 45353506), cultures of five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) and the tryptophan-deficient (*try*$^-$) strain of *Escherichia coli* (WP2 μ*vr*A) were incubated for 48 hours at 37 ± 1.5° C in darkness in the presence of the test substance, CGA 263208, a plant metabolite of CGA 219417 (Batch No. EA168376, 95.2% a.i. in dimethylsulfoxide, DMSO) at five concentrations ranging from 61.73 to 5000 μg/plate in the presence (+S9) or absence (-S9) of hepatic-post mitochondrial supernatant (S9 fraction) from Aroclor 1254-treated young (7 weeks-old) male RAI rats. In addition to cultures exposed only to the vehicle (DMSO), other cultures were treated with strain- specific mutagens. At harvest, the number of revertant colonies (*his*$^-$, *try*$^+$) in test cultures was compared to vehicle controls.

Background growth was reduced only with strain TA100 treated at the highest concentration (5000 μg/plate). However, the numbers of revertant colonies were not reduced with increasing concentration. **In neither trial was there an increase over vehicle controls in the incidence of revertant colonies (*his*$^+$, *try*$^+$) in any test cultures treated at any concentration in the**

**presence or absence of metabolic activation.** Positive control cultures responded with marked increases in revertant colonies.

Therefore, CGA 263208 Technical is negative for reverse gene mutation in these bacterial strains assayed up to the dose limit of testing producing cytotoxicity as evident by some reduction in background lawn.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS:

1. Test Material:
Description: Solid
Lot/Batch No.: EA 168376
Purity: 95.2% a.i.
Stability of compound: Stated to be stable.
CAS No.: Not provided.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: None.

2. Control Materials:

Negative: None.
Solvent/Final concentration: DMSO/0.1 mL/plate.
Positive: **Nonactivation**:
Sodium azide 5.0 µg/plate for TA100, TA1535.
2-Nitrofluorene 20.0 µg/plate for TA98.
9-Aminoacridine 150.0 µg/plate for TA1537.
Other (list): Mitomycin C 2.0 µg/plate for TA102.
4 - NQO[1] 2.0 µg/plate for WP2 µ*vr*A.
**Activation**: 2-Aminoanthracene (2-anthramine) 2.5 µg/plate for TA100, TA98 and TA1537; 20.0 µg/plate for TA102; and 50.0 µg/plate for WP2 µ*vr*A.
Other (list): Cyclophosphamide 400 µg/plate for TA1535.

3. Metabolic Activation: S9 fraction was derived from 7 week-old male RAI rats (Tif: RAIf [SPF]) weighing 177 - 197 g.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| x | Aroclor 1254 | x | induced | x | rat | x | liver |
| | phenobarbital | | non-induced | | mouse | | lung |
| | none | | | | hamster | | other |
| | other | | | | | | other |

[1]4 - NQO, 4 - nitroguinoline - N-oxide.

Describe S9-mix composition:

| Component | Concentration |
|---|---|
| Rat liver S9 fraction | 100.0 µL/mL |
| NADP | 4.0 µM/mL |
| $MgCl_2$ | 8.0 µM/mL |
| KCl | 33.0 µM/mL |
| Na-phosphate-buffer pH 7.4 | 100.0 µM/mL |
| Glucose-6-phosphate | 5.0 µM/mL |

4. Test Organisms: *Salmonella typhimurium* strains:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | TA97 | x | TA98 | x | TA100 | x | TA102 |
| | TA104 | x | TA1535 | x | TA1537 | | TA1538 |

List any others: WP2 µvrA.

Source: Professor B.N. Ames (UCal at Berkeley) for TA98, TA102, TA1535, TA1537; Dr. M.Schűpbach (Hoffmann-La Roche, Basel, SWITZERLAND) for TA100; National Collection of Industrial Bacteria, Aberdeen (SCOTLAND) for WP2 µvrA..

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used.

Cytotoxicity (Range-Finding) Test: (with TA100 and WP2 µ*vr*A ± S9):

20.58, 61.73, 185.19, 555.56, 1666.67, 5000 µg/plate.

Main Assays: (Nonactivated and Activated Conditions):

61.73, 185.19, 555.56, 1666.67, 5000 µg/plate.

B. TEST PERFORMANCE:

A mixture of 2 mL of top agar, 0.1 mL of an overnight culture, plus either 0.5 mL of sodium phosphate buffer (for experiments without activation) or 0.5 mL of S9-mix (for experiments with activation), and 0.1 mL test substance, the positive or solvent control, was poured onto minimal agar in a Petri dish. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). The top agar was composed of 0.6% agar and 0.6% NaCl. The top agar in experiments with *S. typhimurium* was supplemented with 10% 0.5 mM L-histidine and 0.5 mM (+) biotin dissolved in water; with *E. coli* experiments, 10% 0.5 mM L-tryptophan was added.

1. Type of *Salmonella typhimurium* Assay:

| | |
|---|---|
| | Standard plate test |
| | Pre-incubation (___ minutes) |
| | "Prival" modification |
| | Spot test |

Other (describe): Horizontal shaking at 37°C, 130 - 140 rounds/minute.

2. Protocol: Criteria for both assay acceptance and determination of positive responses were presented.

3. Negative and Positive Historical Control Data and Acceptable Ranges for Negative Controls:
Arithmetic Means and SD's of colony counts, obtained previously over 12 months (1993), were presented to confirm the acceptable ranges for mean colony counts of spontaneous revertants.

4. Statistics:

No statistical analyses were performed.

II. REPORTED RESULTS:

A. ANALYTICAL CONTROL:

Values from HPLC analyses were stated to be in agreement with intended concentrations (86.3% and 88.5%), thus said to demonstrate "stability of test substance in the vehicle", but no formal data were presented to support this claim.

B. PRELIMINARY RANGE-FINDING TEST:

Background growth was reduced with TA100 at the HDT (5000 µg/plate +S9). Thus, 5000 µg/plate was selected as the highest concentration ±S9.

C. MUTAGENICITY ASSAYS:

(No cytotoxicity was apparent at any concentration with or without S9 activation.)

In treated cultures of neither the initial nor the confirming assay did the test substance cause significant increases in the incidence of either *his*$^{+}$ or *try*$^{+}$ revertant colonies when compared to negative control (MRID 45353506, pp. 27 to 30 - ATTACHMENT Tables 5 to 8; derived from individual strain data, pp. 31 to 54). All positive controls responded with marked increases in revertants.

Therefore, the investigator concluded that CGA 263208 Technical and its metabolites did not induce gene mutation in the strains of *S. typhimurium* or *E. coli* employed.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator's conclusions that the technical formulation of CGA 263208 was negative for inducing gene mutation (increases in revertant colonies) when assayed in a battery of *Salmonella typhimurium* and *Escherichia coli* strains exposed up to the limit concentration causing moderate cytotoxicity as evident by reduction in the background lawn.

B. STUDY DEFICIENCIES:

None.